

# The *Saccharomyces cerevisiae* SEC14 Gene Encodes a Cytosolic Factor That Is Required for Transport of Secretory Proteins from the Yeast Golgi Complex

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**Abstract.** We have obtained and characterized a genomic clone of *SEC14*, a *Saccharomyces cerevisiae* gene whose product is required for export of yeast secretory proteins from the Golgi complex. Gene disruption experiments indicated that *SEC14* is an essential gene for yeast vegetative growth. Nucleotide sequence analysis revealed the presence of an intron within the *SEC14* structural gene, and predicted the synthesis of a hydrophilic polypeptide of 35 kD in molecular mass. In confirmation, immunoprecipitation experiments demonstrated SEC14p to be an unglycosylated polypeptide, with an apparent molecular

mass of some 37 kD, that behaved predominantly as a cytosolic protein in subcellular fractionation experiments. These data were consistent with the notion that SEC14p is a cytosolic factor that promotes protein export from yeast Golgi. Additional radiolabeling experiments also revealed the presence of SEC14p-related polypeptides in extracts prepared from the yeasts *Kluyveromyces lactis* and *Schizosaccharomyces pombe*. Furthermore, the *K. lactis* SEC14p was able to functionally complement *S. cerevisiae* *sec14<sup>s</sup>* defects. These data suggested a degree of conservation of SEC14p structure and function in these yeasts species.

THE Golgi complex is common to all eukaryotic cells. This organelle is of fundamental importance in the regulation of intracellular protein traffic that transits the later stages of the secretory pathway, the recycling of membrane from the cell surface, and communication with aspects of the endocytic pathway (14). Morphological studies have indicated a conservation of basic Golgi structure throughout the eukaryotic kingdom. The Golgi complex displays a segmented structure consisting of a set of four, or more, membraneous cisternae with flattened centers and dilated rims (9). This structural motif coincides with a compartmentalized biochemical organization that is apparent at several experimental levels. In an effort to identify the Golgi components that mediate protein transport across this organelle, Rothman and colleagues (1, 2, 26, 27) have devised an assay with which vesicular stomatitis virus G protein transport through the Golgi can be followed in vivo and in vitro. These studies have indicated that intercisternal Golgi transport is mediated by vesicles and that cytosolic proteins and ATP are required. There are also indications that fatty acylation reactions and GTP-binding proteins may be involved in this process as well (12, 21).

We have chosen to approach the problem of protein exit from the Golgi from a genetic perspective using the yeast *Saccharomyces cerevisiae* as a model system. The reasons for this are twofold. First, the basic scheme of the yeast secretory pathway is the same as that for mammals. This has

been established by the isolation and characterization of yeast *sec* mutants that exhibit conditional defects at specific stages of the secretory pathway (23). Second, Dunphy et al. (6) have obtained evidence suggesting a measure of functional conservation between yeast and mammals with respect to the cytosolic factors that participate in driving Golgi transport functions in vitro. The yeast *sec* mutant collection identifies two nuclear genes, *SEC7* and *SEC14*, whose products appear to play crucial roles in protein transport through the Golgi (23).

Here we report that invertase secretion was rapidly blocked upon shift of a *sec14<sup>s</sup>* strain to the nonpermissive temperature, suggesting a direct role for SEC14p in facilitating protein transport through yeast Golgi and identifying *SEC14* as a reasonable candidate for detailed molecular analysis. As an initial step toward this goal, we present a characterization of the *SEC14* gene. We show that *SEC14* is an essential gene that belongs to the category of rare *S. cerevisiae* genes that contain introns, and that SEC14p is a rather abundant yeast polypeptide that exhibits no glycosyl modification. Moreover, subcellular fractionation experiments demonstrated SEC14p to behave as a cytoplasmic species, although some association with particulate fractions was also observed. These data are consistent with SEC14p being a cytosolic factor that promotes protein transport through the yeast Golgi complex. Finally, we present evidence to indicate a structural and functional conservation of SEC14p across several yeast species.

We propose that the elucidation of SEC14p function in *S. cerevisiae* will contribute to a general understanding of how proteins exit from the Golgi complex.

## Materials and Methods

### Bacterial and Yeast Strains

*Escherichia coli* strains MC1061 (5) and KK2186, F' *traD36 lac<sup>+</sup>ZAM15 proA<sup>+</sup>B<sup>+</sup>/Δ (lac-pro) supE thi rpsL endA sbcB15 hsdR4*, were used for plasmid maintenance. Yeast haploid strains included YP54 (*MATα ade2-101 ura3-52 Δhis3-200 lys2-801 Δtrp1*) and its isogenic *MATα* derivative, YP52, from R. Davis, and SF292-2A (*MATα sec14-3<sup>+</sup> gal2 mal*) from R. Schekman (University of California). The prototypic *sec<sup>Δ</sup>* strain used in the studies was CTY1-1A (*MATα ura3-52 Δhis 3-200 lys2-801 sec14-3<sup>+</sup>*), a meiotic segregant from SF292-2A × YP54 (this study). Strain CTY182 is the isogenic *SEC14* derivative of CTY1-1A. Yeast diploid strains CTYD1 and CTYD38 were generated by crossing CTY1-1A × YP54 and YP52 × YP54, respectively. Diploid strain CTYD2 is isogenic to CTYD1 and was generated by recombining *sec 14Δ1::HIS3* (see text) into the *SEC14* allele of CTYD1. All strains were constructed by standard genetic techniques (35). Gene disruption technology has been described in principle by Rothstein (28). *Schizosaccharomyces pombe* and *Kluyveromyces lactis* strains SP255 (*h<sup>+</sup> ade 6-210*) and MW98-8C (*MATα arg lys ura*) were obtained from Jo Anne Wise (University of Illinois) and Stephen Johnston (Duke University), respectively.

### Media and Reagents

YP, yeast minimal, and defined minimal media have been described (35). Minimal media for *K. lactis* and *S. pombe* have also been described (22, 25). Standard *E. coli* media were employed (20). All reagents for invertase assays, concanavalin A-Sepharose, protein A-Sepharose, and all protease inhibitors were obtained from Sigma Chemical Co. (St. Louis, MO). Restriction enzymes and nucleic acid modifying enzymes were from Bethesda Research Laboratories (Gaithersburg, MD) and Pharmacia Fine Chemicals (Piscataway, NJ). Reagents for SDS-PAGE, agarose gel electrophoresis, and electrophoresis of DNA sequencing reactions were from Bio-Rad Laboratories (Indianapolis, IN). Trans-Label (>1,000 Ci/mmol) was from ICN Radiochemicals (Irvine, CA).  $\alpha$ -<sup>32</sup>P-dATP (800 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL).

### Invertase Secretion

Strain CTY1-1A was grown to mid-logarithmic phase in YP (2%) glucose medium at 30°C with shaking. Cells were pelleted (2,000 g, 1 min), washed with 2 vol of water, and resuspended in 2 vol of glucose (0.1%) YP medium. The culture was split and incubated as before at 30°C for 30 min. After such time, one culture was shifted to 36°C; the other was kept at 30°C. Samples (500  $\mu$ l) were taken from each culture at 0, 15, 30, 45, and 60 min postshift, immediately adjusted to 10 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, and kept on ice. The samples were washed twice with 1 ml of cold 10 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and resuspended in 500  $\mu$ l of the same. Each sample was subsequently split. For each, the nonpermeabilized sample was adjusted to 500  $\mu$ l with 250  $\mu$ l of 10 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The samples to be permeabilized were similarly adjusted with 10 mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 0.2% Triton X-100 and subjected to one cycle of freeze-thaw. These samples were used to measure external and total invertase activities, respectively, using the assay of Goldstein and Lampen (13). The optical densities of the nonpermeabilized samples were measured at 600 nm with a Gilson spectrophotometer and served to normalize the data. Invertase units were expressed as nanomoles glucose produced per min at 30°C.

### Transformations

*E. Coli* were transformed to ampicillin resistance by the calcium chloride method (20). Yeast were transformed to Ura<sup>+</sup> or His<sup>+</sup> by the lithium acetate procedure (15).

### Cloning and Characterization of SEC14

YCp50 (*SEC14*) plasmids were identified by transformation of CTY1-1A to Ura<sup>+</sup> and, after 24 h of incubation at 30°C, by challenge of the selection plates with nonpermissive growth conditions (36°C). More detailed descriptions of YCp50 are found elsewhere (17, 32). Ura<sup>+</sup> Ts<sup>+</sup> transformants were screened for proper behavior as described in the text. Physical charac-

terization experiments involved subcloning and deletion analyses coupled with complementation tests. The subcloning analyses were performed by introducing defined restriction fragments from the complementing insert of YCp50 (*SEC14*) into the CEN4-URA3 vector pSEY58 (7). Deletion analyses involved the removal of defined fragments from YCp50 (*SEC14*) by restriction with enzymes that cleaved only in the insert DNA (or in some cases also cleaved YCp50 sequences that immediately surround the insert; e.g., Eco RI, Sal I, Sma I), followed by religation. All such manipulations were performed with plasmid DNA purified from *E. coli* and the relevant constructs were confirmed to be correct before reintroduction into yeast for purposes of complementation. Complementation analyses involved transformation of the desired plasmids into CTY1-1A by selection for Ura<sup>+</sup> at 30°C. Purified transformants were screened for their ability to form single colonies at the nonpermissive temperature of 36°C.

### DNA Sequencing

The nucleotide sequence analysis was carried out using the dideoxy chain termination method (33). The inferred SEC14 protein sequence was compared to the Protein Information Resource Library by the FASTP program in both the ktup = 1 and ktup = 2 modes (19).

### SEC14 Antibodies

A phage T4-lysozyme-SEC14p protein fusion was constructed by subcloning a 550-bp Ssp I-Hind III fragment from a *SEC14* deletion construct into the Sna BI-Hind III half-sites of pCKR101. The resultant plasmid (pRE43) programs the synthesis of a 182-residue polypeptide (*M<sub>r</sub>* = 21 kD) in response to the addition of Isopropyl  $\beta$ -D-thiogalactopyranoside to the host growth medium (the T4 lysozyme gene is under pTAC control in this vector system). Residues 1 through 7 of the polypeptide represent the amino terminus of T4 lysozyme, codons 8 and 182 represent fusion codons generated by the construction, and codons 9–181 represent amino acids 111–284 of SEC14p. Codon 183 is an amber codon. Thus, 173 of 182 total residues in the engineered polypeptide represent SEC14p primary sequence. *E. coli* strain MC1061 (pRE43) was found to produce ~10 mg of fusion peptide per liter of cells after a 3-h induction with 2.5 mM IPTG. The hybrid protein was purified via the general procedure reported for TrpE fusion proteins (16) and found to reside in the pellet of a 0.1% SDS, 50 mM Tris (pH 7.5), 50 mM EDTA wash of the insoluble fraction of the total cellular lysate. Antibodies were raised by primary injection of 500  $\mu$ g of hybrid protein into popliteal lymph nodes of New Zealand White female rabbits. After a 6-wk rest, 500  $\mu$ g boost injections were administered intramuscularly every 2 wk until a satisfactory response was obtained.

### Immunoprecipitation of SEC14 and CPY

Strain CTY182 was grown to an early logarithmic growth stage (OD<sub>600</sub> = 0.5) in 2% glucose yeast minimal medium lacking methionine and cysteine. A 2-ml aliquot of the culture was radiolabeled with Trans-Label (150  $\mu$ Ci/ml) for 30 min at 30°C. Incorporation of label was terminated by the addition of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to 10 mM and the culture was split into five equal aliquots. Protein was TCA precipitated from all samples, followed by cell lysis with glass beads, boiling in 1% SDS buffer, clarification of extract by centrifugation, and dilution of extract in immunoprecipitation buffer that had 0.5% Tween 20 substituted for 2% Triton X-100 (34). Four of these yeast extracts were treated with CPY antiserum and either (a) preimmune SEC14p serum, (b) immune SEC14p antiserum, (c) SEC14p antiserum plus 50  $\mu$ l of cell-free extract prepared from *E. coli* MC1061 (pRE43) not induced for T4 lysozyme-SEC14p fusion protein synthesis, and (d) SEC14p antiserum plus 50  $\mu$ l of extract derived from MC1061 (pRE43) cells induced for lysozyme-SEC14p fusion protein synthesis. These *E. coli* extracts represented protein obtained from two OD<sub>600</sub> units of cells. The remaining yeast extract was treated with 30  $\mu$ l packed beads of concanavalin A-Sepharose and separated into bound and soluble fractions (34). Each fraction was treated with CPY and SEC14p antisera, and resolution of the immunoprecipitates was achieved by SDS-PAGE and autoradiography as described (34). CPY and SEC14p antibodies were added in a 1:1,000 final dilution whereas the preimmune serum was added in a 1:500 final dilution.

### Detection of SEC14p Cross-Reacting Species

*S. cerevisiae* CTY182, *K. lactis* MW98-8C, *S. pombe* SP255, and *S. cerevisiae* CTY1-1A/pKLI2 (see text) were grown to an OD<sub>600</sub> of 0.5 in their respective glucose minimal media, to which appropriate nutritional supplements had been added (22, 25, 35) at 30°C with shaking. One OD<sub>600</sub> unit

of cells from each yeast culture was radiolabeled with Trans-Label (150  $\mu$ Ci) for 30 min at 30°C. Recovery of immunoprecipitates from clarified extracts of TCA precipitates with SEC14p antiserum and preimmune serum were performed exactly as described above.

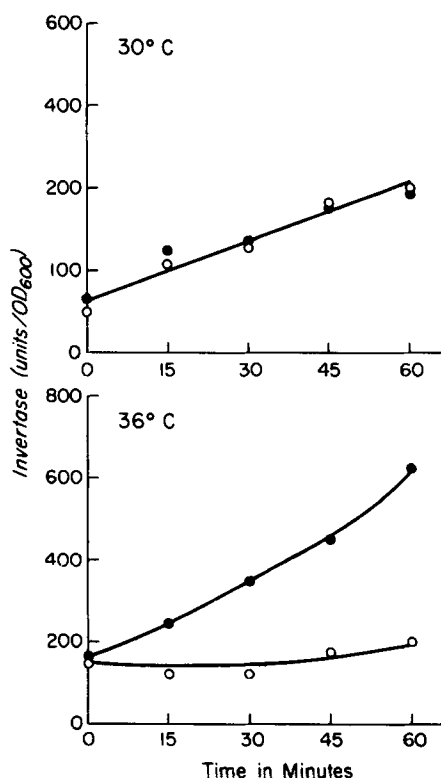
### Fractionation of SEC14p

Strain CTY182 was grown to an OD<sub>600</sub> of 0.5 in glucose defined minimal medium with shaking at 22°C. Some five OD<sub>600</sub> units of cells were radiolabeled with 50  $\mu$ Ci of Trans-Label per OD<sub>600</sub> cells for 20 min at 22°C. Incorporation of label was terminated by the addition of NaN<sub>3</sub> to 10 mM. The cells were washed with 1 ml of 10 mM NaN<sub>3</sub> and converted to spheroplasts by resuspension in spheroplast buffer (50 mM KPO<sub>4</sub> [pH 7.5], 1.4 M sorbitol, 50 mM  $\beta$ -mercaptoethanol, 10 mM NaN<sub>3</sub>), addition of 2.5  $\mu$ g/OD<sub>600</sub> cells of oxalyticase (Enzogenetics, Corvallis, OR), and incubation at 30°C for 15 min. This treatment resulted in virtually quantitative conversion of yeast cells to spheroplasts. Spheroplasts were pelleted by a 2-min spin at 500 g, and resuspended in 1.5 ml of lysis buffer (10 mM Mops [pH 7.5], 0.3 M sorbitol, 2  $\mu$ g/ml chymostatin, leupeptin and pepstatin A, 1 mM PMSF, 0.1 mM EGTA, 3 mM benzamide, 30 U/ml aprotinin, and 100  $\mu$ g/ml BSA). 500  $\mu$ l of the lysate were removed and saved as the whole cell extract. The remaining 1.0 ml was first centrifuged for 3 min at 500 g to remove intact cells and other large debris, yielding the low speed pellet, and the resulting supernatant was spun at 12,000 g for 15 min at 4°C. The low speed and 12,000 g pellets were solubilized in 150  $\mu$ l of boiling buffer (50 mM Tris, pH 7.5, 1% SDS, 1 mM EDTA). The 12,000 g supernatant fraction was centrifuged for 1 h at 100,000 g in a rotor (model SW 50.1; Beckman Instruments, Inc., Palo Alto, CA) and an ultracentrifuge (model L8-70 M; Beckman Instruments, Inc.) to generate the high speed supernatant and high speed pellet fractions. The high speed pellet was solubilized in 150  $\mu$ l of boiling buffer. TCA was used to precipitate protein from the high speed supernatant and the whole cell fractions and the precipitates were also solubilized in 150  $\mu$ l of boiling buffer. Samples (15  $\mu$ l) of clarified extracts derived from the whole cell, low speed pellet, 12,000 g pellet, high speed supernatant, and 100,000 g pellet fractions were treated with CPY, SEC14p, or invertase antibodies. Precipitates were analyzed by SDS-PAGE and autoradiography as described above.

## Results

### Invertase Secretion Responds Rapidly to Loss of SEC14p Function

The classification of *secl4<sup>ts</sup>* mutants as being conditionally defective in Golgi transport processes is based upon the observation that such yeast exhibit a marked exaggeration of Golgi-related structures (Berkeley bodies) under nonpermissive conditions, and that the intracellular invertase pool that accumulates under such restrictive conditions consists of enzyme that exhibits fully matured outer glycosyl chains (8, 23). Previous analyses of the secretion competence of *secl4<sup>ts</sup>* yeast incubated under nonpermissive conditions involved the measurement of intracellular invertase and acid phosphatase accumulation after a rather long exposure of the cells to the restrictive temperature (23). To assess the rate at which the *secl4<sup>ts</sup>* secretory block is imposed, we adopted an experimental approach described by Salminen and Novick (32) that allowed us to compare the efficiency of invertase secretion in *secl4<sup>ts</sup>* yeast to the differential rate of invertase synthesis. CTY1-1A (*secl4<sup>ts</sup>*, *SUC2*) cells were grown at 30°C in YP medium containing a final glucose concentration of 2%; a condition that represses secretory invertase synthesis. The cells were shifted to 0.1% glucose YP medium and incubated at 30°C for 30 min to allow for induction of secretory invertase synthesis. The culture was subsequently split into two equal portions, with one portion incubated at 30°C and the other at 36°C. Samples were collected at various times post-shift from each, and total and extracellular invertase activities were determined (see Materials and Methods). As is evi-



**Figure 1.** Invertase secretion is rapidly blocked upon imposition of the *secl4<sup>ts</sup>* defect. Strain CTY1-1A (*secl4<sup>ts</sup>*, *SUC2*) was cultured in YP medium containing 2% glucose at 30°C. The cells were shifted to YP (0.1% glucose) medium for 30 min at 30°C, the culture was split, and one portion was incubated at 30°C and the other at 36°C. At the indicated times after temperature shift, aliquots were removed and total and extracellular invertase was determined for each. ○, extracellular invertase levels; ●, total invertase activities. All points represent the averages of three determinations that in all cases deviated from each other by <10%. This figure depicts a representative experiment and consistent results were obtained in other experimental trials.

dent from the data shown in Fig. 1, CTY1-1A cells incubated at the permissive temperature (30°C) exhibited an extracellular distribution for essentially all of the invertase that was synthesized after shift (i.e.,  $t = 0$ ). This point is indicated by the observation that the total invertase activity, measured for Triton X-100 permeabilized yeast, was entirely accounted for by the cell surface invertase measured in parallel yeast samples that had not been permeabilized. Different results were obtained for CTY1-1A cultures incubated at 36°C. Even at the early 15-min time point essentially all of the invertase synthesized postshift was detected in a latent form. This result was also observed for the 30-, 45-, and 60-min time points. Parallel experiments with the isogenic *SEC14* strain, CTY 182, indicated the absence of any significant intracellular accumulation of invertase at 30 or 36°C (not shown). These data indicated that invertase secretion was rapidly blocked upon shift of the *secl4<sup>ts</sup>* strain to the restrictive temperature.

### Isolation of the SEC14 Gene

The recessive nature of *secl4<sup>ts</sup>* allowed recovery of *SEC14* clones from existing DNA libraries by complementation.

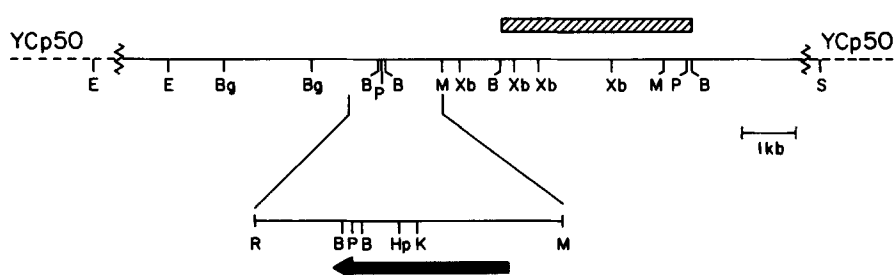
Strain CTY1-1A (*MAT $\alpha$* , *ura3-52*,  $\Delta$ *his3-200 lys2-80I*, *secl4-3<sup>ts</sup>*) was transformed with a yeast genomic DNA library maintained in YCp50, a shuttle plasmid carrying a yeast centromere and the *URA3* gene. The desired transformants were obtained by simultaneous selection for uracil prototrophy and temperature resistance (see Materials and Methods). From an estimated  $2 \times 10^4$  potential Ura<sup>+</sup> transformants, 20 were recovered that were also Ts<sup>+</sup>. Linkage of the Ts<sup>+</sup> character to the plasmid was tested by curing each of the candidate transformants of their resident YCp50 plasmids by selecting for five fluoroorotic acid-resistant derivatives (3). Six of the candidate transformants yielded ts<sup>-</sup> derivatives upon such curing events and, by this criterion, the original six Ura<sup>+</sup> Ts<sup>+</sup> transformants were considered to identify putative *SEC14* clones. The cognate plasmids were recovered from these candidates and purified. Retransformation of these purified plasmids back into CTY1-1A, selecting for Ura<sup>+</sup>, confirmed the acquisition of Ts<sup>+</sup> as an unselected marker in all cases. Restriction mapping experiments demonstrated that all six complementing plasmids contained overlapping segments of the yeast genome. A detailed physical characterization of one such plasmid insert (pCTY3) was performed (Fig. 2). By a combination of deletion mapping and subcloning analyses, the complementing activity of the pCTY3 insert was localized to a 2.0-kb Mlu I-Eco RV restriction fragment. Such experiments also indicated that the Kpn I, Hpa I, and Pvu II sites were all internal to the *secl4<sup>ts</sup>* complementing gene; as were the two Bam HI sites (Fig. 2). Various internal regions of the presumed *SEC14* gene were employed as templates from which radiolabeled RNAs of known polarity were generated by run-off transcription in vitro, and used to probe total and polyadenylated yeast RNA fractions. These Northern analyses revealed a unique hybridizing RNA species of some 1.2 kb in length that was observed in both total and poly A<sup>+</sup> RNA fractions, and was transcribed in the direction of Mlu I towards Bam HI. This mRNA was found to be approximately as abundant as the *TRP1* transcript at the steady state level (Cleves, A., and V. Bankaitis, unpublished data).

To prove that the complementing activity of pCTY3 represented the authentic *SEC14* gene, two independent genetic criteria were employed. In the first case, a 3.6-kb Bam HI fragment that lies adjacent to *SEC14* (see Fig. 2) was subcloned into the integrating vector YIp5 (4). Integration of the recombinant plasmid (pCTY14) into the genomic homolog of the 3.6-kb Bam HI fragment was directed by linearization

of pCTY14 at the unique Mlu I site of the insert and subsequent transformation of CTY1-1A (*secl4<sup>ts</sup>*) to Ura<sup>+</sup>. The resultant haploid strain (CTY175) was verified to have experienced the expected integration event as judged by meiotic segregation analysis of *URA3* and by Southern analysis of genomic DNA prepared from it (data not shown). CTY175 was mated to YP54 (*MAT $\alpha$* , *ura3-52*, *SEC14*), the resultant diploid was induced to sporulate, and the meiotic progeny were examined by standard tetrad analysis. For the total of 30 asci that were dissected, all tetrads were found to yield four viable spores and to exhibit an absolute cosegregation of *secl4<sup>ts</sup>* and the YIp5-borne *URA3* marker. These data indicated that the complementing gene of pCTY3 originated from a region of the yeast genome that is homologous to the *SEC14* locus. The second criterion demanded that recombination of an in vitro-generated disruption of the complementing gene into the yeast genome result in a disruption of *SEC14*. To construct such a disruption, a 5.5-kb Eco RI-Xba I restriction fragment was subcloned from pCTY3 (Fig. 2) into the corresponding sites of pUC18 (38). The yeast *HIS3* gene was then used to replace a 168-bp Bam HI fragment lying within the Eco RI-Xba I insert, resulting in a disruption of the complementing gene by deletion and insertion mutagenesis. The purified Eco RI-Xba I fragment was used to transform a diploid strain, CTYD1 (*MAT $\alpha$ /MAT $\alpha$* ,  $\Delta$ *his3-200/His3-200*, *sec 14<sup>ts</sup>/SEC14*), to His<sup>+</sup>. Of 200 His<sup>+</sup> transformants examined, 97 were found to exhibit the recessive *secl4<sup>ts</sup>* phenotype. The simplest explanation for these data was that a *SEC14* disruption had been recombined into the wild-type *SEC14* allele of the diploid test strain. Southern analysis of genomic DNA prepared from several representative His<sup>+</sup>ts<sup>-</sup> transformants verified that the expected recombination events had occurred. These results, when coupled with the integrative mapping data, proved the authenticity of the cloned *SEC14* gene. The particular disruption allele that we have described here is designated *secl4 $\Delta$ 1::HIS3*. As described below, *secl4 $\Delta$ 1::HIS3* represents a deletion of sequences encoding the carboxy-terminal end of the *SEC14* protein.

### *SEC14* Encodes an Essential Function

The conditional phenotype of *secl4<sup>ts</sup>* yeast may result from synthesis of a temperature-sensitive gene product or, alternatively, might describe a *secl4* null phenotype. To distinguish these possibilities *secl4 $\Delta$ 1::HIS3* was recombined into the



**Figure 2.** Physical map of the yeast *SEC14* gene. The ~13-kb genomic insert of the *secl4<sup>ts</sup>* complementing plasmid pCTY3 (see text) is given. The *SEC14* coding region is defined by the heavy arrow, which also indicates the direction of *SEC14* transcription (see text). Restriction site abbreviations are as follows: Eco RI (E), Bgl II (Bg), Bam HI (B), Pvu II (Pv), Mlu I (M), Xba I (Xb), Eco RV (R), Hpa I (Hp), Kpn I (K), and Sal I (S). The 3.5-kb Bam HI fragment that was used for the integrative genetic mapping experiments is indicated by the hatched bar (see text).

*SEC14* gene of the yeast diploid strain CTYD1 (see above). The resultant diploid (CTYD2) was then subjected to tetrad analysis. Dissection of a total of 113 tetrads revealed three classes of asci. The predominant class consisted of 109 tetrads that uniformly yielded two viable spores and two inviable spores. The other classes were each represented by two asci that yielded three viable spores and one inviable spore per ascus, and one viable and three inviable spores per ascus, respectively. All 226 viable haploid progeny were phenotypically His<sup>-</sup> and, therefore, received *sec14<sup>u</sup>* and not *sec14Δ1::HIS3*. No His<sup>+</sup> progeny were obtained, suggesting that *SEC14* is an essential yeast gene. The minority tetrad classes provided strong support for this conclusion as these presumably identified gene conversion events at the *SEC14* locus and strengthened the correlation between *sec14Δ1::HIS3* and lethality. The parental diploid (CTYD1) exhibited excellent spore viabilities upon tetrad analysis.

To further verify that the lethal consequences of receiving *sec14Δ1::HIS3* were not due to some trivial aspect of the specific construction, a second disruption of *SEC14* was constructed. This was accomplished by inserting *HIS3* into the unique Hpa I site that resides within the *SEC14* structural gene, resulting in preservation of only the amino-terminal one-third of the SEC14 protein (see below). The new disruption was recombined into one of the *SEC14* alleles of a diploid yeast strain (CTYD38) that is homozygous for *Δhis3-200* and *SEC14*. Analysis of 30 tetrads from the *SEC14::HIS3/SEC14* derivative showed that all 30 asci yielded two viable progeny apiece and that all of these 60 surviving haploid segregants were His<sup>-</sup>. These results were in agreement with those obtained from the first cross, and confirmed the essential nature of *SEC14*.

Microscopic visualization of the inviable spores obtained from the first cross indicated that the spores had all germinated. In most cases, the null mutants completed one to two rounds of cell division before cessation of growth. Thus, we concluded that *SEC14* encodes a function that is essential for yeast vegetative growth.

### *SEC14* Nucleotide Sequence

The localization of *SEC14* to a 2.0-kb Mlu I–Eco RV restriction fragment, coupled with the identification of marker restriction sites within the *SEC14* gene (Fig. 2), facilitated determination of the *SEC14* nucleotide sequence (Fig. 3). We interpreted the sequence data to indicate that *SEC14* encodes a polypeptide of 304 residues ( $M_r = 35,032$ ) that is terminated by a single UGA codon. This polypeptide was inferred to be of a hydrophilic nature (27% charged amino acids) and to exhibit a net negative charge at neutral pH ( $pI = 5.3$ ). Hydropathy analysis suggested that only two regions of the inferred primary sequence exhibited net hydrophobic character. These regions were bounded by residues 165 and 194 (inclusive), and residues 212 and 245 (inclusive), respectively (Fig. 4). The longest uninterrupted stretch of uncharged residues was 14 and extended from GLY181 to VAL194 of the SEC14p primary sequence. As no obvious structure resembling a classical signal peptide was discerned, it seemed unlikely that SEC14p was itself a passenger of the secretory pathway. We also noted that SEC14p did not display any potential sites for N-linked glycosyl modification.

Our interpretation of the SEC14p sequence demanded the excision of a 156 nucleotide intron from that portion of the

*SEC14* mRNA that encodes the extreme amino terminus of the gene product. As illustrated in Fig. 3, a consensus 5' splice site (5'CTATGT3'; reference 24) immediately followed what we indicated as the third *SEC14* codon. This was followed by a second canonical splicing sequence (5TACTAAC3') that was observed 105 nucleotides downstream from the consensus 5' splice site. The TACTAAC box is known to participate in the formation of the lariat splicing intermediate (37). Normally, the 3' splice site is defined by the first AG encountered that is at least nine nucleotides downstream from TACTAAC. For *SEC14*, the first AG was encountered only eight nucleotides downstream from TACTAAC, and a second AG was observed 36 nucleotides downstream from TACTAAC. If the latter AG marked the 3' end of the intron, the predicted splicing event would preserve the reading frame defined by the indicated initiator codon (Fig. 3). This reading frame would then extend through the restriction sites that have been genetically defined to reside within *SEC14* (e.g., Kpn I, Hpa I, Pvu II, and the two Bam HI sites). Sequence analysis of a *SEC14* cDNA clone confirmed the authenticity of the intron, and that the second AG defined the 3'-splice junction (Malehorn, D. E., and V. A. Bankaitis, unpublished data). We noted that this was the only open reading frame of significant length that was revealed by the sequence analysis, and that the next available in-frame AUG corresponded to MET75. We also noted that the Bam HI sites lay at the extreme 3'-end of the *SEC14* coding region and that the Hpa I site sat about one-third of the way into the *SEC14* coding sequence. As described above, interruption of *SEC14* sequence at either of these regions resulted in lethal dysfunction of SEC14p. Comparisons of the *SEC14* nucleotide sequence, and inferred primary sequence to the GenBank and Protein Information Resource Bank data bases failed to reveal homologies of obvious significance.

### Identification of SEC14p

Our interpretation of the *SEC14* nucleotide sequence data predicted a molecular mass of 35 kD for the gene product. Furthermore, the data indicated that SEC14p would not exhibit asparagine-linked glycosyl modifications. To subject these predictions to the most direct test, a 20-kD fragment of SEC14p was engineered for high-level production in *E. coli*, partially purified, and used as a source of antigen against which a polyclonal antiserum was raised in rabbits (see Materials and Methods). This antiserum was then used to specifically precipitate SEC14p from radiolabeled yeast extracts for analysis. To visualize SEC14p, strain CTY182 (*SEC14*) was radiolabeled for 30 min with [<sup>35</sup>S]methionine and [<sup>35</sup>S]cysteine. Incorporation of label was terminated with NaN<sub>3</sub> and TCA, and SEC14p was precipitated from clarified extracts and Con A–Sepharose bound and unbound fractions derived from such extracts. CPY was also precipitated in these experiments for two purposes. First, it served as a reference to which SEC14p levels could be normalized. Second, it served as a control glycoprotein for Con A binding experiments. The immunoprecipitates were evaluated by SDS-PAGE and autoradiography.

As the data in lane B of Fig. 5 indicate, treatment of radiolabeled CTY182 extracts with both CPY and SEC14p antisera precipitated two major radiolabeled species; the 61-kD mCPY and a polypeptide of some 37 kD in molecular mass. Trace amounts of the CPY precursor forms were also ob-

-370 -360 -350 -340 -330 -320 -310 -300 -290 -280  
 ACAGCGTGAATAT CTTCCTCATT AATACCAATT GCATTATTAC CAGGGGCTTG TTCTATATCA CCAGTATCAA AAAGGGAAC TCTCCCTAGT GAACCAATGT  
 -270 -260 -250 -240 -230 -220 -210 -200 -190 -180  
 ATGGTGGCGT ATCAACCAAT ATCCCTGACC TTACATCCAC CTTTGGGTC AAATAGTGA CACATTAGGA ATAAAAATA TGAAATCATG TTTATTTACG  
 -170 -160 -150 -140 -130 -120 -110 -100 -90 -80  
 TGTTAGATGT TCCGTACAAC GCGACTGGCT AGTTCGTTT TTCCACATT TTTTTCCTCA CGTTGATCAA CAATAAAATG GTACATCTTC CTTTGTGTGT  
 -70 -60 -50 -40 -30 -20 -10 15  
 GTCTTAAGTG TCCTTTCCGT GTATATCGCT TGACCAGCGC CTGTAGCATC AAACCAAAAC TTCATCGCCC TAAAAACA ATG GTT ACA GTATGT  
 MET Val Thr / Intron  
 25 30 45 55 65 75 85 95 105 115  
 TGTTGCTTTT ATTACTTTT TCTTTTITG ACATTCAATG TGACAATAT CACATCTTC AGATAGTCT GTCTATATGA AGCAAAAATG ATATATCAAT  
 125 135 145 155 165 175 180 185 195 205  
 AAGTTTACTA ACAAAACAAA GTGTATTAC TATGACTTCA CTTTAAATAG CAA CAA GAA AAG GAA TTT TTA GAA TCC TAC CCT CAA AAC  
 / Gln Gln Glu Lys Glu Phe Leu Glu Ser Tyr Pro Gln Asn  
 210 220 230 240 250 260 270 280 290 300  
 TGT CCT GAT CCA GGT GGT ACT CCA GGA AAT TTA GAC AGC GCT CAA GAG AAG GCA TTG GCA GAA CTA AGA AAA CTT TTG  
 Cys Pro Pro Asp Ala Leu Pro Gly Thr Pro Gly Asn Leu Asp Ser Ala Gln Glu Lys Ala Leu Ala Glu Leu Arg Lys Leu Leu  
 310 320 330 340 350 360 370 380 390 400  
 GAA GAC GCT GGT TTC ATT GAA CGT TTA GAC GAT TCA ACT TTA CTA CGT TTT TTG AGA GCC AGA AAA TTT GAT GTT CAA TTG GCT  
 Glu Asp Ala Gly Phe Ile Glu Arg Leu Asp Asp Ser Thr Leu Leu Arg Phe Leu Arg Ala Arg Lys Phe Asp Val Gln Leu Ala  
 410 420 430 440 450 460 470 480 490 500  
 AAA GAA ATG TTT GAA AAC TGC GAA AAA TGG AGG AAG GAT TAT GGT ACC GAC ACT ATC TTG CAA GAT TTT CAT TAT GAT GAA AAA  
 Lys Glu Met Phe Glu Asn Cys Glu Lys Trp Arg Lys Asp Tyr Gly Thr Asp Thr Ile Leu Gln Asp Phe His Tyr Asp Glu Lys  
 510 520 530 540 550 560 570 580 590 600  
 CCA TTG ATT GCC AAA TTC TAC CCA CAA TAT TAT CAT AAA ACC GAT AAA GAT GGC CGC CCA GTA TAT TTT GAA GAA TTA GGT GCT  
 Pro Leu Ile Ala Lys Phe Tyr Pro Gln Tyr His Lys Thr Asp Lys Asp Gly Arg Pro Val Tyr Phe Glu Glu Leu Gly Ala  
 610 620 630 640 650 660 670 680 690 700  
 GAT AAC TTA CAT GAA ATG AAC AAG GTT ACC TCT GAA GAG AGG ATG TTG AAA AAC TTG GTT TGG GAA TAC GAA TCT GTC GTT CAA  
 Val Asn Leu His Glu Met Asn Lys Val Thr Ser Glu Glu Arg Met Leu Lys Asn Leu Val Trp Glu Tyr Glu Ser Val Val Val Gln  
 710 720 730 740 750 760 770 780 790 800  
 TAC AGA TTA CCT GCC TGT TCA AGA GCT GGT CAC CTA GTG GAA ACT TCA TGT ACA ATT ATG GAT TTG AAA GGT ATC TCC ATA  
 Tyr Arg Leu Pro Ala Cys Ser Arg Ala Ala Gly His Leu Val Glu Thr Ser Cys Thr Ile Met Asp Leu Lys Gly Ile Ser Ile  
 810 820 830 840 850 860 870 880 890 900  
 TCT AGT GCA TAC AGT GTT ATG TCA TAT GTT AGG GAA GCC TCC TAC ATA AGT CAA AAC TAT TAC CCC GAA CGT ATG GGT AAA TTT  
 Ser Ser Ala Tyr Ser Val Met Ser Tyr Val Arg Glu Ala Ser Tyr Ile Ser Gln Asn Tyr Tyr Pro Glu Arg Met Gly Lys Phe  
 910 920 930 940 950 960 970 980 990 1000  
 TAC ATC ATC AAC GCG CCA TTC GGT TTC TCT ACC GCA TTT AGG CTA TTT AAA CCT TTC TTG GAT CCA GTC ACT GTT TCA AAG ATT  
 Tyr Ile Ile Asn Ala Pro Phe Gly Phe Ser Thr Ala Phe Arg Leu Phe Lys Pro Phe Leu Asp Pro Val Thr Val Ser Lys Ile  
 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100  
 TTT ATC TTG GGT TCT TCT TAC CAG AAG GAA TTA TTA AAG CAA ATT CCA GCT GAA AAC TTA CCA GTC AAA TTT GGC GGT AAG TCT  
 Phe Ile Leu Gly Ser Ser Tyr Gln Lys Glu Leu Lys Lys Gln Ile Pro Ala Glu Asn Leu Pro Val Lys Phe Gly Gly Lys Ser  
 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200  
 GAA GTT GAT GAA TCC AAG GGT GGG TTA TAC CTA TCC GAT ATC GGT CCA TGG AGC GAT CCA AAG TAT ATT GGA CCG GAA GGT GAA  
 Glu Val Asp Glu Ser Lys Gly Gly Leu Tyr Leu Ser Asp Ile Gly Pro Trp Arg Asp Pro Lys Tyr Ile Gly Pro Glu Gly Glu  
 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300  
 GCT CCG GAA GCC TTT TCG ATG AAA TGA TATTATTTTAAAGACATTTTTTTTTCGAGAGAGAAAAG AGGAGTTCTA AGTAACGATA TTTTACAACT  
 Ala Pro Glu Ala Phe Ser Met Lys \*\*\*  
 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400  
 GAGATAAGTA CAAGTATGCT TATTACCTCT ATTCTACTTT TTGTAATAGG CATAGTTTAC ACTTTTATCT CCTTAGTTTG TACTACTCTT TTTTATTTT  
 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500  
 TTTTTCCTT TCCACTCAGC TATTAGATTA TTTTATTTT TATTATCTGT GGTATCATTT TCTACAGCCA TACCTTGATT TTAGTTTATT TTGTGGCAG  
 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600  
 TTATATACAT TTATCTTATA GAATACATAI GCACGTATTA AACAATAAAA AGAACATAAG CAAAAATGAA ACCTCGCTAC AATAAAACTT TATTACGCA  
 1610 1620 1630 1640 1650 1660 1670 1680 1690 1700  
 TGCACTGCGC CGTAAAGAGT ATTCATTAGA AGTACAATGS TAGCCCTATT TGGTTGCAAC ATGTTTATAGC AAAGAGATTA GTAGGCTAGG ATATCAAGTC  
 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800  
 CATGCCCTTT TATCACATAC TGTAATGATA GATGCACTCC GGAAGAATAA AAAAAGTTT TCAGGTTTAC ATTGTGCAAA TTGCGAGTCT ATCTCGAATA  
 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900  
 ATTTTGAGC GCTCATTTCA CGGTTGAGCC GTTTGTATC ACAAGCCAAG TTTAACATTT TATTTTAACA GGGTTCACCG AATTATATTC CCAAAATTGCT  
 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000  
 AATCTTTTAC AATTATGCT TTTGTCTTC TCTCTGAACCTCTCTG

Figure 3. Nucleotide sequence of *SEC14*. The DNA sequence of the *SEC14* coding strand and the predicted primary sequence of its product are shown. The intron is bounded by the hash marks and is indicated by the dotted underline. Hallmark restriction sites that are referred to in the text are shown.

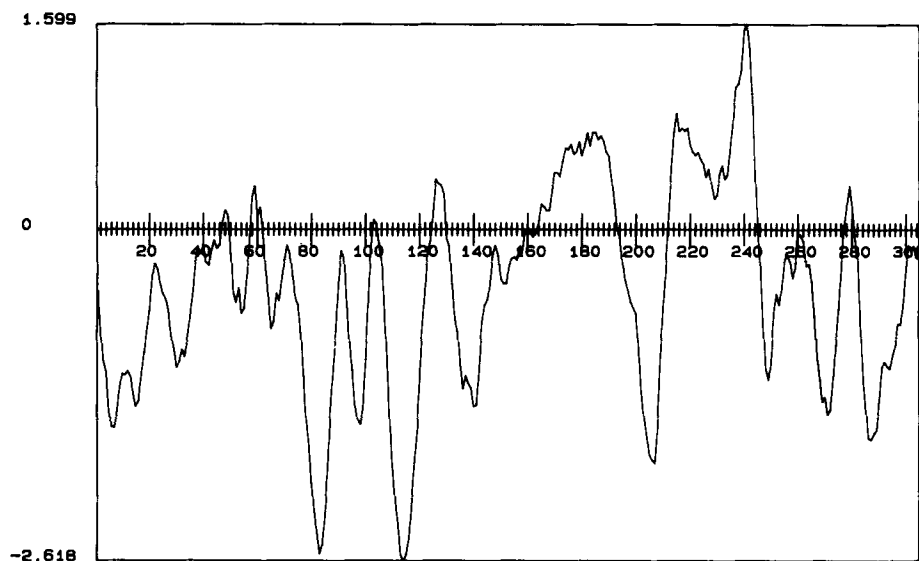
served. The 37-kD species was not apparent when preimmune serum was substituted for SEC14p antiserum in the precipitation reaction (lane A). Furthermore, precipitation of this polypeptide was not competed by the inclusion of unlabeled extract from *E. coli* cells that were not induced for synthesis of the 21-kD SEC14p antigen (lane E), but was effectively competed by inclusion of extract from *E. coli* cells that were induced for such antigen synthesis (lane F). This competition experiment, when coupled with the data obtained with the preimmune serum, identified the 37-kD polypeptide as the yeast SEC14p. Pulse-chase experiments have indicated SEC14p to be as stable as mCPY in yeast cultures undergoing logarithmic growth (manuscript in preparation). Thus, densitometric estimations of the relative levels of SEC14p and mCPY suggested that these species were present at similar intracellular concentrations (within a factor of two; data not shown).

As shown in Fig. 5, lane C, the Con A bound fraction from

CTY182 extracts yielded only the CPY immunoreactive species. No SEC14p was recovered from this fraction. The 37-kD SEC14p was quantitatively recovered from the Con A supernatant fraction (lane D) and, as expected, no CPY species were recovered from this soluble fraction. Taken together, these data demonstrated SEC14p to be a rather abundant and unglycosylated polypeptide of 37 kD in apparent molecular mass. These data also provided strong confirmation for our interpretation of the sequence data; especially with respect to assignment of the initiator codon (Fig. 3).

### SEC14p Resides Primarily in the Cytosolic Fraction

The nucleotide sequence data indicated SEC14p to be of hydrophilic character and suggested a cytoplasmic location for this polypeptide. To test this directly, we employed standard differential centrifugation techniques to fractionate radiolabeled yeast lysates into various membrane enclosed and cyto-

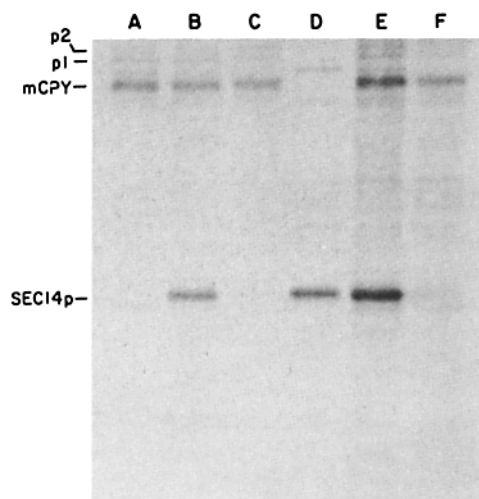


**Figure 4.** Hydropathy analysis of SEC14p. The inferred SEC14p amino acid sequence was used to generate a hydropathy profile by the method of Kyte and Doolittle (18). A scanning window of seven residues was used. Amino acid residues are numbered along the horizontal axis while net hydrophobicity values are indicated on the vertical axis. Hydrophilic values lie below the horizontal axis while hydrophobic values lie above.

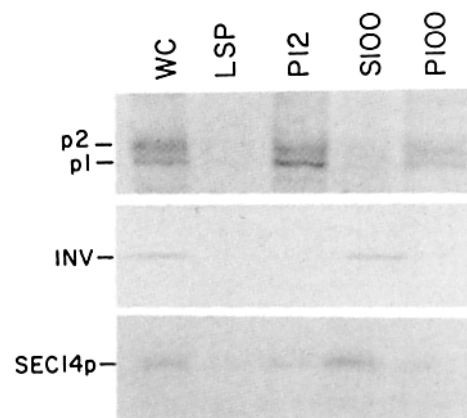
plasmic compartments (see Materials and Methods). The presence of SEC14p in these various fractions was determined by quantitative immunoprecipitation analysis of each fraction with SEC14p antiserum. The osmotic lysis procedure we employed was very similar to those previously reported (10, 29), and it was expected that membrane-enclosed

organelles would remain intact upon cell lysis. The conditions of radiolabeling were designed to reveal the CPY as the p1 and p2 forms of the enzyme. These precursor forms of CPY were employed as luminal markers of ER and early Golgi complex, and late Golgi complex, respectively (36). The constitutively expressed form of invertase was employed as a cytoplasmic marker.

From the data shown in Fig. 6, it is clear that the p1 and p2 CPY sedimented quantitatively with the 12,000 g and



**Figure 5.** Identification of SEC14p. Strain CTY182 (*SEC14*) was grown in glucose (2%) yeast minimal medium and allowed to incorporate radiolabel for 30 min at 30°C. Clarified extracts were recovered and immunoprecipitations performed with CPY antiserum and variable SEC14p sera: lane A, CPY antiserum and preimmune SEC14p serum; lane B, CPY antiserum and immune SEC14p serum. Lanes C and D represent precipitations of CPY and SEC14p from Con A bound and Con A supernatant fractions derived from the clarified extract, respectively. Lanes E and F indicate the profiles obtained when the same clarified extract was treated with CPY and SEC14p antibodies either in the presence of *E. coli* extract derived from cells not induced for SEC14p antigen synthesis (lane E), or in the presence of *E. coli* extract obtained from cells induced for high-level synthesis of the SEC14p antigen against which the polyclonal SEC14p antiserum was raised (lane F). The precursor and mature forms of the glycoprotein CPY are indicated at left, as is the SEC14p.



**Figure 6.** Fractionation of SEC14p to the yeast cytosol. Strain CTY182 was grown in minimal defined glucose (2%) medium, radiolabeled with Trans-Label, osmotically lysed, and the lysate subjected to three rounds of differential centrifugation (see Materials and Methods). The whole cell (WC), 500 g pellet (LSP), 12,000 g pellet (P12), 100,000 g supernatant (S100), and 100,000 g pellet (P100) fractions were probed for the presence of precursor forms of CPY, the cytoplasmic form of invertase, and SEC14p. The p1 and p2 CPY, indicated at left, served as luminal markers for ER and early Golgi complex, and late Golgi complex, respectively. Cytoplasmic invertase served as a cytoplasmic marker. All of the immunoprecipitates displayed here were recovered from aliquots of the same clarified lysates. Note that the WC fraction was derived from one-half of the material that was used to generate the other fractions (see Materials and Methods). We estimate our recoveries of antigen after fractionation to exceed 80%.



100,000 g pellet fractions and exhibited an ~60 and 40% distribution of material between these two pellet fractions, respectively. The virtual absence of soluble p1 and p2 CPY indicated that the integrity of ER and Golgi complex had been satisfactorily maintained by the lysis procedure. In contrast, radiolabeled invertase was recovered exclusively from the 100,000 g supernatant fraction, as expected for a soluble and not membrane enclosed species. An identical fractionation profile was observed for phosphoglycerate kinase, another cytoplasmic species (data not shown). We also noted the significant absence of cytoplasmic invertase and luminal proCPY forms in the low speed pellet fraction, suggesting that quantitative lysis of spheroplasts had been achieved.

SEC14p exhibited a fractionation profile that was most similar to that of cytoplasmic invertase. In the experiment shown, some 65% of the radiolabeled SEC14p was recovered from the 100,000 g supernatant fraction. However, some 35% of the total, radiolabeled SEC14p cosedimented with material that pelleted at 500, 12,000, and 100,000 g (Fig. 6). The proportion of SEC14p that was observed to sediment in the pellet fractions varied somewhat with the method of cell lysis and from experiment to experiment (not shown). We have reproducibly observed a range of 20–40%. The significance of these associations with pellet are presently unclear as we cannot exclude the possibility that the sedimentable SEC14p is artifactually retained in these fractions. We note that essentially no invertase or phosphoglycerate kinase was observed to pellet under either of these conditions. The conclusion derived from these data is that the predominant fraction of SEC14p was localized to the yeast cytosol.

### SEC14 Epitopes Are Conserved in Widely Divergent Yeasts

Dunphy et al. (6) have obtained compelling evidence indicating that *S. cerevisiae* and mammals employ functionally interchangeable cytosolic factors to drive intercisternal Golgi transport in vitro. These data suggest that elements of structural conservation may exist between eukaryotic components involved in essential Golgi functions. To test whether SEC14p exhibits such a structural conservation in widely divergent organisms, the polyclonal SEC14p antiserum was employed as a specific probe for the presence of SEC14p homologs in extracts prepared from other yeast species. These included the budding yeast *Kluyveromyces lactis* and the fission yeast *Schizosaccharomyces pombe*. *S. cerevisiae* CTY182, *K. lactis* MW98-8C, and *S. pombe* SP255 were radiolabeled and incorporation of label was terminated with TCA. Clarified extracts from each yeast strain were prepared and split into two equal aliquots. One aliquot was treated with preimmune serum while the other was mixed with SEC14p antiserum. The antibody complexes were washed under stringent conditions and evaluated by SDS-PAGE and autoradiography.

As shown in Fig. 7, treatment of the yeast extracts with preimmune serum failed to precipitate any radiolabeled species. Although only the relevant portion of the autoradiogram is shown, the preimmune serum lanes were blank in all three cases. However, the SEC14p antiserum did precipitate a single protein from all three yeast extracts. Not only was the expected 37-kD polypeptide recovered from *S. cerevisiae* extracts, but a 39-kD immunoreactive species was detected in *K. lactis* extracts, and a 35-kD protein was precipi-

tated from *S. pombe* extracts. The *K. lactis* and *S. pombe* SEC14p immunoreactive polypeptides were specifically recognized by the SEC14p antibodies since, in both cases, this binding was quantitatively competed by the 21-kD T4 lysozyme-SEC14p fusion protein against which the SEC14p antiserum was raised (data not shown). We noted that *K. lactis* and *S. pombe* incorporated only one-third and one-seventh of the total radiolabel that was incorporated by *S. cerevisiae* strain CTY182, respectively. Sample loadings were normalized on the basis of such incorporation values. We emphasize that, by this criterion, the *S. pombe* extract was underrepresented by a factor of two with respect to the *S. cerevisiae* and *K. lactis* extracts used in the immunoprecipitation experiment shown in Fig. 7. Consequently, the SEC14p antiserum appeared to recognize the *K. lactis* and *S. pombe* polypeptides with approximately equal efficiencies. These data demonstrated that both *K. lactis* and *S. pombe* exhibited polypeptides that were authentically related to the *S. cerevisiae* SEC14. Since these yeasts span evolutionary distances that are as great between *S. cerevisiae* and *S. pombe* as those between either of these yeasts and mammals (30), it is clear that SEC14p epitopes are conserved across wide phylogenetic boundaries.

Do the elements of structural conservation of SEC14p underlie a functional conservation as well? In an initial effort to address this question, attempts were made to isolate genomic clones of *K. lactis* and *S. pombe* genes that could complement, or suppress, the *sec14-3<sup>s</sup>* mutation of CTY1-1A. We were immediately successful in isolating several such clones from a *K. lactis* genomic library propagated in the yeast shuttle vector YEp24 (generously provided by J. Salmeron and S. Johnston; references 28, 31). The details will be described elsewhere. Radiolabeling of CTY1-1A bearing a representative complementing *K. lactis* clone (pKL12), followed by precipitation of SEC14p immunoreactive materials from cell-free extracts and resolution of the products by SDS-PAGE and autoradiography, yielded the profile shown in Fig. 7. Clearly, two major SEC14p species and a minor SEC14p species were observed. The SEC14p of lowest apparent molecular mass, a major species, migrated in a fashion that identified it as the *S. cerevisiae* SEC14p. The doublet migrating at 39–40 kD in apparent molecular mass represented the SEC14p antigenic homolog from *K. lactis*. We do not know why this doublet was observed when the *K. lactis* SEC14p was expressed in *S. cerevisiae* but, apparently, not in *K. lactis*. We noted that synthesis of the *K. lactis* SEC14p as a doublet was common to all three independent *sec14<sup>s</sup>* complementing clones that have been obtained (not shown). These results suggested that, at least in the case of *K. lactis*, the structural conservation of SEC14p epitopes underlies some conservation of protein function as well, and that the *K. lactis* SEC14p could substitute for that of *S. cerevisiae*.

A trivial explanation for functional rescue of the *sec14<sup>s</sup>* defect could be imagined in a situation where the *K. lactis* SEC14p and the *S. cerevisiae* Sec14p<sup>s</sup> interact. Such an interaction might then make the defective Sec14p<sup>s</sup> more efficient. If this were the case, one would not expect the *K. lactis* SEC14 to overcome the haploid lethal phenotype of an *S. cerevisiae* *sec14* null mutation. To test this directly, pKL12 was transformed into CTYD2 (*MATa/MAT $\alpha$ , ura3-52/ura3-52,  $\Delta$ his3-200/ $\Delta$ his3-200, *sec14<sup>s</sup>/sec14 $\Delta$ 1::HIS3*) by linear transformation to Ura<sup>+</sup>. The resultant diploid was then subjected to tetrad analysis. Of the 10 tetrads analyzed, we found seven*



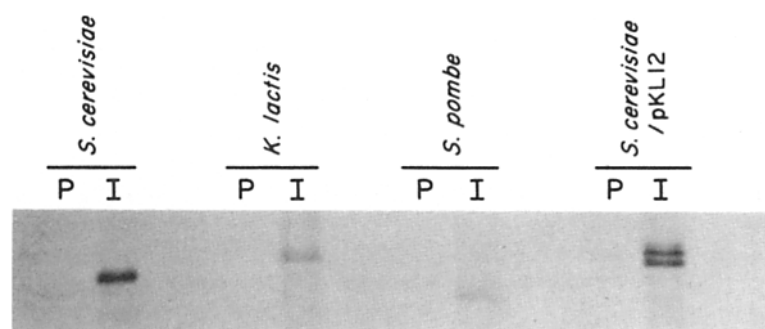


Figure 7. SEC14p homologs from *K. lactis* and *S. pombe*. *S. cerevisiae* CTY182, *K. lactis* MW98-8C, *S. pombe* SP255, and *S. cerevisiae* CTY1-1A (*sec14<sup>ts</sup>*) carrying a *K. lactis* genomic DNA clone (pKL12) that complements the *sec14<sup>ts</sup>* lesion (see text), were cultured in their respective glucose minimal media at 30°C and radiolabeled with Trans-Label for 30 min. Clarified extracts were prepared from each culture and split. One aliquot was treated with preimmune SEC14p serum (P), while the other was treated with SEC14p antiserum (I). Immunoprecipitates were resolved by SDS-PAGE and autoradiography. Sample loadings were normalized as a function of total incorporation of radiolabel into TCA-precipitable counts. By virtue of its rather poor incorporation of radiolabel, the *S. pombe* extract was underrepresented, relative to the other extracts, by a factor of two in the immunoprecipitation reactions.

to yield four viable Ura<sup>+</sup> haploid progeny. Each of these seven tetrads also exhibited a 2 His<sup>+</sup>/2 His<sup>-</sup> segregation pattern (data not shown). As we have established that *sec14-Δ1::HIS3* is a haploid lethal mutation (see above), these data demonstrated that pKL12 suppressed the *sec14* disruption allele. Further confirmation for this conclusion was obtained from experiments where haploid *S. cerevisiae* strains carrying pKL12 were shown to successfully receive *sec14Δ1::HIS3* or *SEC14::HIS3* (see above) by linear transformation, and execute proper replacement of *SEC14* with these null alleles. Taken together, these data demonstrated directly that the *K. lactis* SEC14p was functionally homologous to the *S. cerevisiae* SEC14p.

## Discussion

The classification of *sec14<sup>ts</sup>* mutants as being defective in Golgi transport function is based on both cytological and biochemical data (8, 23). These include the observation that *sec14<sup>ts</sup>* yeast exhibit a marked exaggeration of Golgi-derived structures under nonpermissive conditions, and that the intracellular pool of secretory glycoprotein that accumulates under such conditions consists of polypeptides that exhibit fully matured outer chain glycosyl modifications. Although such data constitute persuasive evidence for an involvement of SEC14p in yeast Golgi function, these data do not allow one to distinguish whether the involvement is of a direct or an indirect nature.

We have obtained evidence to support a direct participation of SEC14p in yeast secretory processes. Invertase secretion was demonstrated to have been dramatically blocked upon shift of *sec14<sup>ts</sup>* yeast to restrictive conditions. Furthermore, this secretory block was imposed with rapid kinetics (Fig. 1). A rapid and marked inhibition of invertase secretion seems more likely to represent a primary defect in yeast secretory function, rather than the manifestation of some secondary effect resulting from a more general metabolic difficulty. Secondary effects might reasonably have been expected to exhibit a longer course of onset, and a less specific phenotype in terms of organelle exaggeration.

To gain some insight into the role of SEC14p in yeast secretory metabolism, we have isolated and characterized the *SEC14* gene from a *S. cerevisiae* genomic library. Genetic proof for

the authenticity of the clone was obtained from integrative mapping and gene disruption experiments. The gene disruption experiments also indicated *SEC14* to be an essential gene. Nucleotide sequence analysis of *SEC14* identified a 1,071-bp structural gene with the potential of encoding a 304-amino acid polypeptide of mol wt = 35,032 (Fig. 3). This was in good agreement with Northern blot analyses that sized the *SEC14* mRNA at ~1.2 kb. An interesting feature of the *SEC14* sequence was the clear presence of a 156-bp intron at the extreme 5'-end of the structural gene. Very few of the *S. cerevisiae* genes whose nucleotide sequences have been determined exhibit introns. Those that do, with a single exception, exhibit only one intron and it is found at the extreme 5'-end of the gene (11). In this respect, the *SEC14* intron was typical. Examination of the inferred SEC14p primary sequence revealed an acidic polypeptide of hydrophilic character that lacked any obvious signals for membrane insertion or entry into the yeast secretory pathway (Fig. 4). Comparisons of the *SEC14* nucleotide sequence and inferred SEC14p sequence to the GenBank and PIR data bases failed to reveal any homologies of obvious significance that might have provided some clue as to the biochemical nature of SEC14p function.

Some general insight as to possible functions for SEC14p are provided by fractionation experiments. We have identified the *S. cerevisiae* SEC14p to be an unglycosylated polypeptide of some 37 kD in apparent molecular mass (Fig. 5). These determinations were in excellent agreement with those predicted by the nucleotide sequence. Furthermore, the *S. cerevisiae* SEC14p was found to be quite an abundant intracellular species; approximately as abundant as the vacuolar CPY. Fractionation of radiolabeled yeast lysates indicated SEC14p to primarily reside in the 100,000 g supernatant fraction (Fig. 6). As a result, models requiring a luminal or integral membrane disposition for SEC14p are rejected. The present data suggest that SEC14p represents a cytosolic factor that is involved either in promoting intercisternal transport of secretory polypeptides through the Golgi Complex or, alternatively, in facilitating the packaging of secretory protein into the appropriate vesicle carrier for final delivery. The requirement for cytosolic factors in driving vesicle-mediated transport of protein through the Golgi complex is well-documented (1, 2, 6, 26, 27). The significance of the pelletable forms of SEC14p remains to be determined.

Although the precise biochemical mechanism for SEC14p function remains elusive, we have obtained evidence to suggest a conservation of SEC14p structure. Such evidence was derived from experiments where polyclonal antisera directed against *S. cerevisiae* SEC14p were employed as probes for homologs in widely divergent yeast species. Immunoprecipitations of SEC14p cross-reactive materials from *K. lactis* and *S. pombe* extracts have identified polypeptides of 39 and 35 kD in apparent molecular mass, respectively, that behaved as authentic homologs of the *S. cerevisiae* SEC14p (Fig. 7). This is a rather remarkable result considering the wide divergence between *S. cerevisiae* and *S. pombe* (30). At least in the case of *K. lactis*, the observed antigenic homology underlay a functional homology as well. We demonstrated that genomic clones of *K. lactis* DNA that complemented the *S. cerevisiae* *sec14<sup>ts</sup>* defect directed the synthesis of the *K. lactis* SEC14p (Fig. 7). Since the *S. pombe* SEC14p did not appear to be any less cross-reactive with *S. cerevisiae* SEC14p than was the *K. lactis* homolog, we predict that the *S. pombe* SEC14p will also prove to be functionally homologous. Experiments are currently underway to test this hypothesis.

Is a measure of structural and functional conservation of SEC14p likely to be observed only among divergent yeasts? Preliminary Southern blot analyses of mammalian and fish genomic DNAs have identified potential *SEC14*-related nucleic acid sequences in these organisms as well as in *S. pombe* (not shown), a yeast for which we have already demonstrated the existence of an antigenic homolog (Fig. 7). Although we have, to this point, failed to unequivocally demonstrate a SEC14p cross-reactive polypeptide in mammalian Vero cell extracts (data not shown), these data are intriguing in light of the work of Dunphy et al. (6) who showed a functional interchangeability between *S. cerevisiae* and mammals with respect to the cytosolic factors that are required to drive intercompartmental Golgi transport in vitro. We have generated conditions where it is possible to remove functional SEC14p activity from yeast lysates. With the in vitro transport assays firmly in place, such SEC14p-depleted cytosolic fractions can now be tested for Golgi transport-promoting activity, with the hope of obtaining direct evidence for SEC14p involvement as a cytosolic, Golgi transport-promoting factor. We believe that continued genetic and biochemical analysis of SEC14p function will reveal important insights as to how intracellular protein traffic proceeds through the later stages of the eukaryotic secretory pathway.

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